−0.9964. By using this slope in the equation $E_a = -4.58$ (slope), an E_a of 14,922 cal/mol was calculated for this reaction. Correlation coefficients for the other five reactions of BuOH/SBO, 30:1 catalyzed by 1% H_2SO_4 were −0.9918, −0.9965, −0.8918, −0.9795 and −0.9905.

Energies of activation determined for other reactions in our study ranged from 8,000-20,000 cal/mol (Table 3). Other investigators have reported E_a values within this range for other transesterification reactions (6,8,9-12). E_a for the shunt reaction TG-GL had a value of 20 kcal/mol. The spread of values seen in Table 3 for E_a is due partly to experimental error. In addition, the simplified model used in the equation $\log_{10}K = (-E_a/2.30R)/T + C$ may not be adequate to account for all the variables involved. This might also explain why E_a for some reverse reactions are greater than those of the corresponding forward reactions. Further research is needed to develop a model that might provide a narrower range of E_a values.

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Study on the Oxidative Rate and Prooxidant Activity of Free Fatty Acids

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Oleic, linoleic and linolenic acids were autoxidized more rapidly than their corresponding methyl esters. Addition of stearic acid accelerated the rate of autoxidation of methyl linoleate and the decomposition of methyl linoleate hydroperoxides. Therefore, the higher oxidative rate of FFA's than their methyl esters could be due to the catalytic effect of the carboxyl groups on the formation of free radicals by the decomposition of hydroperoxides. Addition of stearic acid also accelerated the oxidative rate of soybean oil. This result suggests that particular attention should be paid to the FFA content that affects the oxidative stability of oils.

A few papers (1,2) have been published on the comparison of the oxidative rate of free fatty acids (FFA) and their esters. Holman et al. (2,3) reported that FFA's were oxidized more rapidly than their esters, and he suggested that this effect probably was due to participation of the carboxyl groups in the decomposition of peroxides. He also said in this review (3) that this idea has been supported by the work of Privett et al., in which addition of linoleic acid to methyl linoleate peroxide accelerated its decomposition. However, it was not clear whether its decomposition was promoted by the catalytic action of the carboxyl group in linoleic acid or oxidized products of this acid, and the effect of FFA's on the autoxidation of esters has not been investigated.

In this paper, we report the detailed data for the difference in oxidative rates between FFA's and their methyl esters with periodic measurement of the unoxidized substrate content by GLC and POV, and elucidate the catalytic action of the carboxyl group in FFA's on the autoxidation of oils by the use of stearic acid as a catalyst which is not autoxidized under the conditions of the present experiment.

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MATERIALS AND METHODS

Preparation of materials. Oleic, linoleic and linolenic acids

were prepared from olive, safflower and linseed oils, respectively. The mixed methyl esters, obtained from each vegetable oil by transesterification using sodium methoxide as the catalyst, were separated by the method of urea adduction (4) and $AgNO_3$-silicic acid column chromatography (5). These purified methyl esters were saponified and unsaponifiable matters, such as tocopherols, were removed. After acidifying the solution, the FFA's were extracted with diethyl ether. The recovered FFA's were refined by silicic acid column chromatography before use.

Methyl oleate, linoleate and linolenate were prepared from oleic, linoleic and linolenic acids purified by the method described above. The conversion to methyl esters was carried out with 7% BF_3-methanol. Methyl esters were refined by silicic acid column chromatography before use.

Each refined FFA and its methyl ester gave only a single spot on thin layer chromatography (TLC), and the refined methyl ester showed a purity exceeding 99% by gas liquid chromatography (GLC).

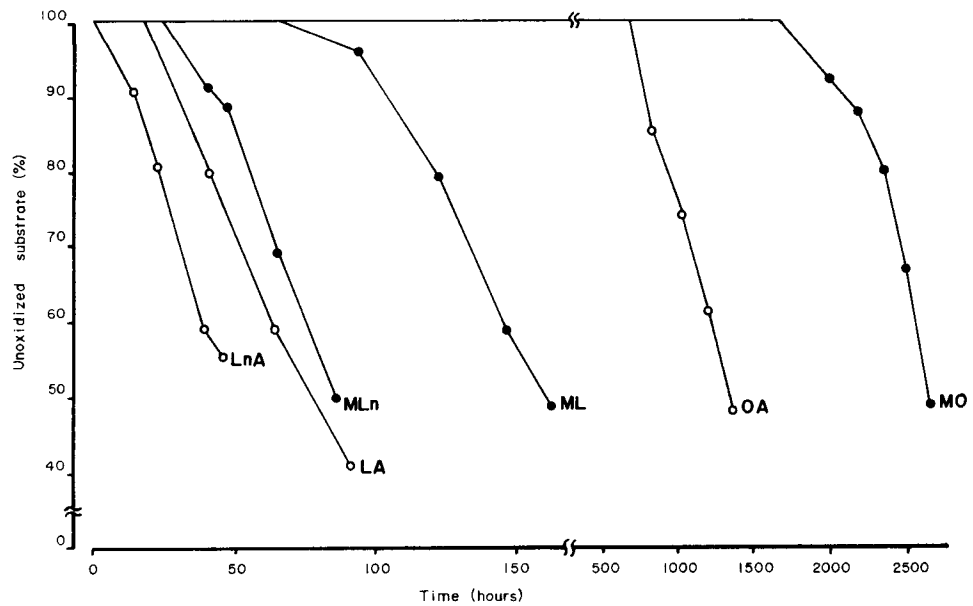

FIG. 2. Changes in the amount of unoxidized substrate during autoxidation at 50 C. LnA, linolenic acid; LA, linoleic acid; OA, oleic acid; MLn, methyl linolenate; ML, methyl linoleate; MO, methyl oleate. The values are represented as the percentage of starting materials.

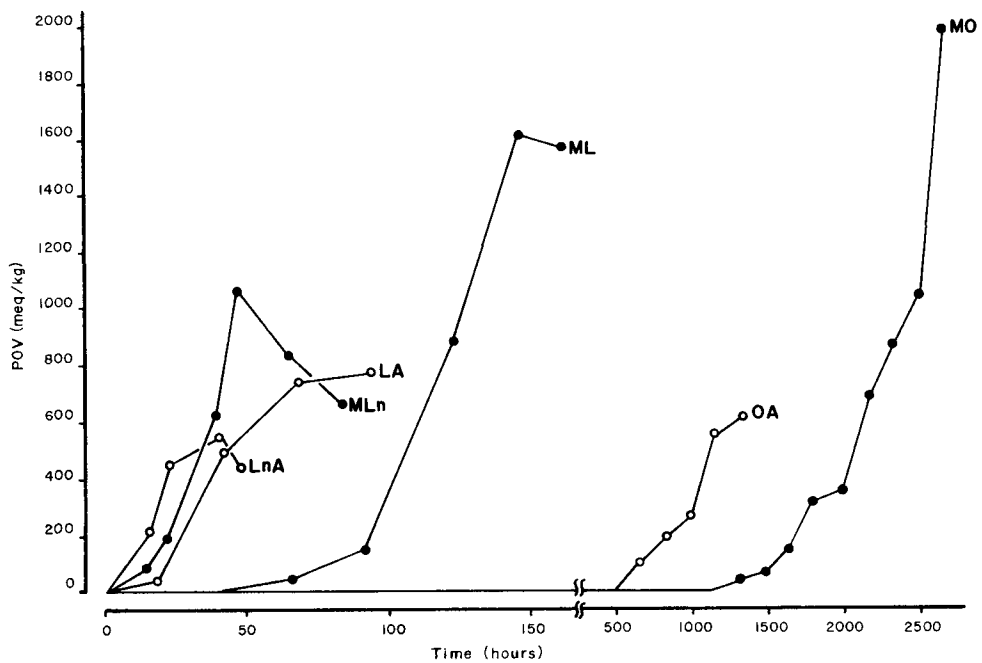

FIG. 1. Changes in POV of free fatty acids and their methyl esters during autoxidation at 50 C. LnA, linolenic acid; LA, linoleic acid; OA, oleic acid; MLn, methyl linolenate; ML, methyl linoleate; MO, methyl oleate.

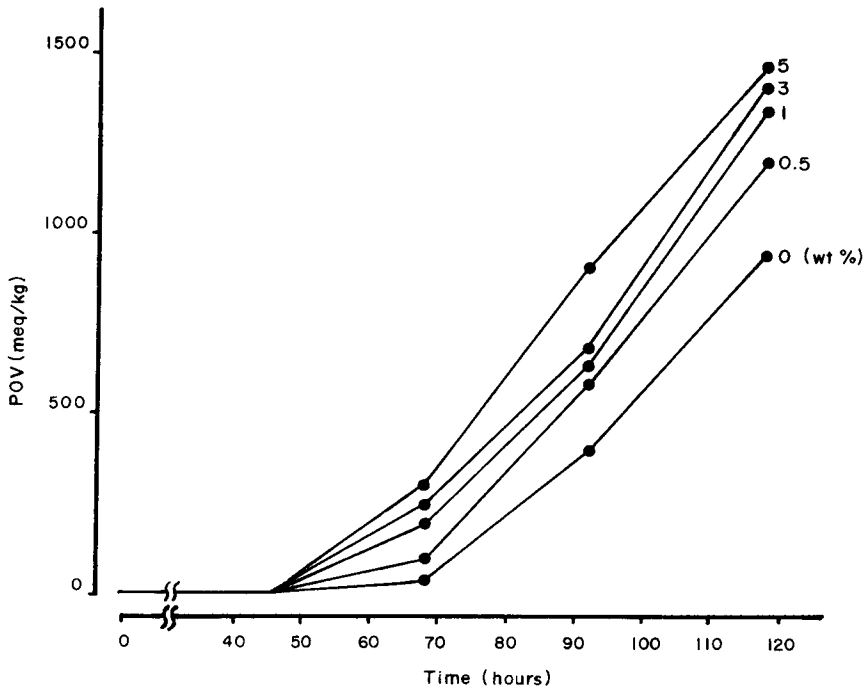

FIG. 3. Effect of stearic acid on the autoxidation of methyl linoleate: Changes in POV.

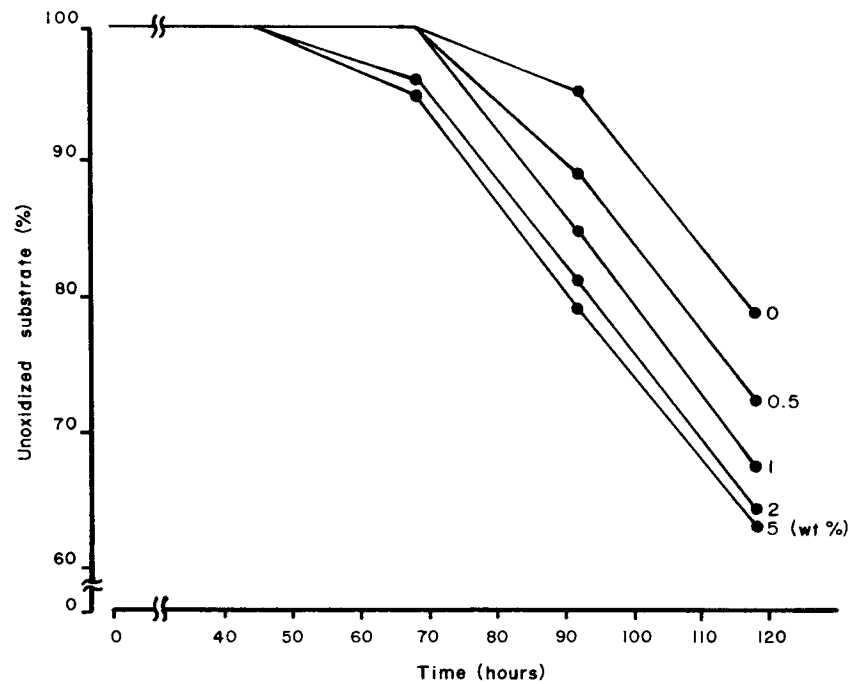

FIG. 4. Effect of stearic acid on the autoxidation of methyl linoleate: Changes in the amount of unoxidized substrate. The values are represented as the percentage of starting materials.

Soybean oil was obtained from manufacturers and purified by silicic acid column chromatography to remove trace amounts of impurities such as hydroperoxides, tocopherols and FFA's.

Oxidation procedure. One g of FFA's or their methyl esters in a flat-bottomed glass tube (30 ml, 3 cm id) was autoxidized by incubation in the dark at 50 C. An aliquot of the sample was taken for determination of POV and for GLC at certain time intervals. POV was determined by the colorimetric iodine method (6). The content of unreacted substrate in an autoxidized sample was determined by the following method using GLC. An autoxidized sample was accurately weighed. After addition of a known amount of methyl laurate as an internal stan-

dard, the methyl ester sample was subjected to silicic acid column chromatography to remove oxidized products, and then to GLC. The FFA sample was converted to methyl ester with 7% BF_3-methanol after addition of methyl laurate, and then subjected to silicic acid column chromatography and to GLC. Contents of the unreacted material were calculated from the peak ratios of the methyl esters to an internal standard. GLC was carried out with a Shimadzu GC-6AM apparatus (Shimadzu Seisakusho Co. Ltd., Kyoto) equipped with dual flame ionization detectors and glass columns (150 × 0.4 cm) packed with 10% DEGS on Chromosorb-WAW. The column temperature was 170 C. The detector and injector temperature were maintained at 230 C. The carrier gas was nitrogen, and it had a flow rate of 30 ml/min.

Effects of stearic acid on autoxidation of methyl linoleate (ML) and soybean oil. One g of ML or soybean oil containing 0 to 5 wt% (for ML) or 0 to 1 wt% (for soybean oil) of stearic acid (purity above 98% in GLC) was autoxidized under the same conditions as described above. The change of POV and the decrease of unoxidized substrates during autoxidation also were determined as described above.

Effects of stearic acid on decomposition of methyl linoleate hydroperoxides (MLHPO). MLHPO were separated from autoxidized ML (POV:1700) with column chromatography on a silica gel 60 column (3 × 40 cm) by developing with n-hexane/diethyl ether (7). These MLHPO were found to contain no secondary products by TLC on which they gave only a single spot. The purity of these MLHPO was confirmed by POV measurement (6170 meq/kg, theoretical value: 6135). After addition of stearic acid to the MLHPO at a level corresponding to 0 to 1 wt% of the substrate, 0.5 g of MLHPO in a flat-bottomed glass tube (6 ml, 1.5 cm id) was incubated under the atmosphere and in the dark at 50 C, and the change of POV and conjugated diene was measured. The amount of conjugated diene was calculated from the determination of absorbance at 233 nm, according to Banks et al. (8). The UV absorption was measured in methanol with a Shimadzu UV-160 spectrophotometer (Shimadzu Seisakusho Co. Ltd., Kyoto).

RESULTS AND DISCUSSION

Figure 1 shows the changes in POV of FFA's and their methyl esters during autoxidation. Judging from the POV changes of FFA's, the highest oxidative rate was shown by linolenic acid, followed by linoleic and oleic acids. In the case of their esters, methyl linolenate was found to be most susceptible to autoxidation, followed by methyl linoleate and oleate. The POVs of FFA's were definitely higher than those of their corresponding methyl esters at the same reaction period in the initial stage of autoxidation.

Changes in the amount of unoxidized substrate during autoxidation (Fig. 2) also demonstrated that the oxidative rate increased markedly as the unsaturation increased, and that FFA's were autoxidized more rapidly than their methyl esters. The induction period for autoxidation of FFA's and their methyl esters under the conditions of this study were judged by the results of unoxidized substrate contents as follows: oleic acid, 700 hr; linoleic acid, 22 hr; linolenic acid, 10 hr; methyl oleate, 1860 hr; methyl linoleate, 91 hr, and methyl linolenate, 34 hr.

Since the main reason for the difference in oxidative rates of FFA's and their corresponding methyl esters could be due to carboxyl groups in FFA's (3), the oxidative rate of ML was compared with that of ML containing stearic acid, which is extremely stable to autoxidation under the same conditions (Figs. 3,4). The POV changes in ML, with various concentrations of stearic acid (0–5 wt%), are shown in Figure 3. The oxidative rate of ML increased with increasing concentrations of stearic acid. This prooxidative effect of stearic acid on the autoxidation of ML was confirmed by the determination of the unoxidized substrate contents (Fig. 4). In the GLC analysis, methyl laurate was used as an internal standard. It was also shown that stearic acid contents of samples did not decrease as autoxidation proceeded. These results

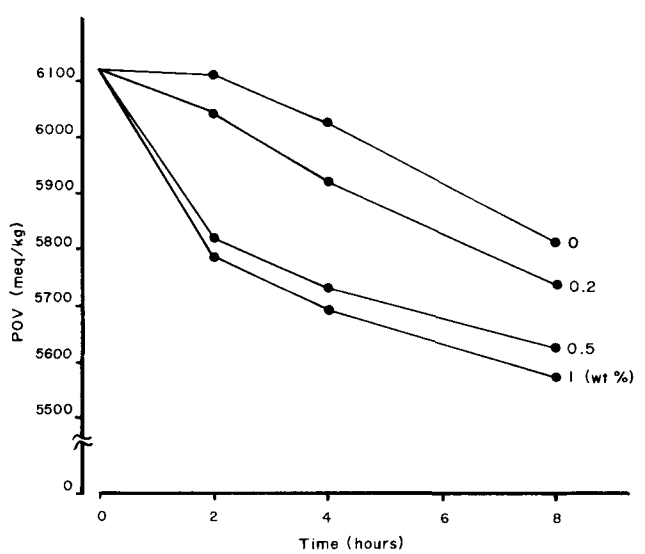

FIG. 5. Effect of stearic acid on the decomposition of methyl linoleate hydroperoxides: Changes in POV.

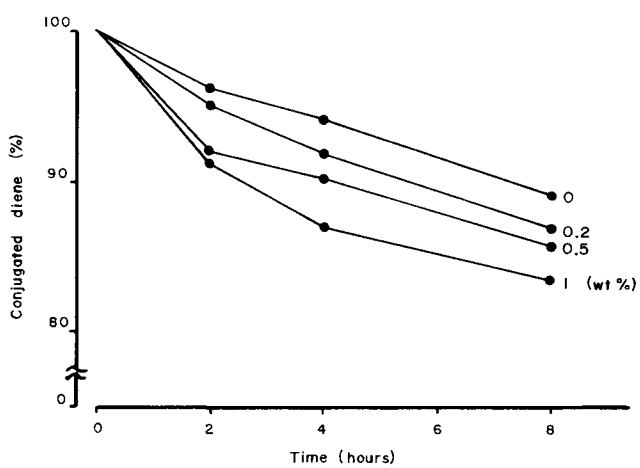

FIG. 6. Effect of stearic acid on the decomposition of methyl linoleate hydroperoxides: Changes in the content of conjugated diene. The values are represented as the percentage of starting materials.

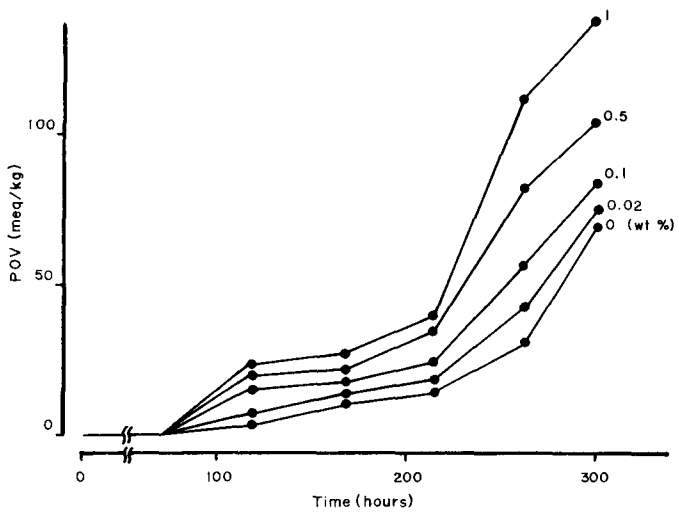

FIG. 7. Effect of stearic acid on the autoxidation of soybean oil: Changes in POV.

suggested that the autoxidation of ML was accelerated by the carboxyl group of stearic acid, while stearic acid was not autoxidized.

To elucidate the mechanism for this catalytic action of carboxyl groups on the autoxidation of ML, the effect of stearic acid on the stability of MLHPO was studied. Figures 5 and 6 show the changes of POV and conjugated diene content of MLHPO with various concentrations of stearic acid. The addition of stearic acid accelerated the decrease of POV (Fig. 5) and conjugated diene content (Fig. 6). These results indicated that the decomposition of MLHPO accompanied by the destruction of conjugated diene structure was promoted by the addition of stearic acid. Free radicals formed by the decomposition of MLHPO were presumed to promote the autocatalytic oxidation of ML. Therefore, it is reasonable that the higher oxidative rate of FFA's compared to their methyl esters is due to the catalytic action of carboxyl groups in FFA's on the decomposition of a small amount of hydroperoxides formed in the initial stage of autoxidation.

The first step of the decomposition of an unsaturated hydroperoxide is generally known to be the homolytic cleavage of the oxygen-oxygen bond or oxygen-hydrogen bond to yield alkoxy and hydroxy radicals or peroxy and hydrogen radicals (9-11). This homolytic decomposition is catalyzed by Fe(II), Fe(III), Fe(III)-cysteine (12-15), hemoglobin (16), radicals (17,18) and others. On the other hand, treatment of hydroperoxides with acid catalyst such as H_2SO_4 (19,20) and HCl (21) results mainly in heterolytic cleavage of the hydroperoxy group, and no radicals are formed from the acid heterolytic decomposition. The decomposition of hydroperoxides stimulated by FFA's presumably occurs homolytically, because stearic acid catalyzed the decomposition of MLHPO and the decomposition was presumed to accelerate autoxidation by radical chain reaction.

Figure 7 shows the POV changes of soybean oil containing various concentrations of stearic acid (0-1 wt%). The prooxidant activity of stearic acid on the autoxidation of soybean oil was observed at a concentration of 0.02 wt%, which corresponded to 0.04 of acid value. The oxidative rate of soybean oil increased markedly with increasing concentrations of stearic acids. Therefore, it is necessary to pay attention to the FFA contents of oils, though many factors affected the oxidative stability of oils.

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